

Figure 1A: Full length Intron A = 820 bp

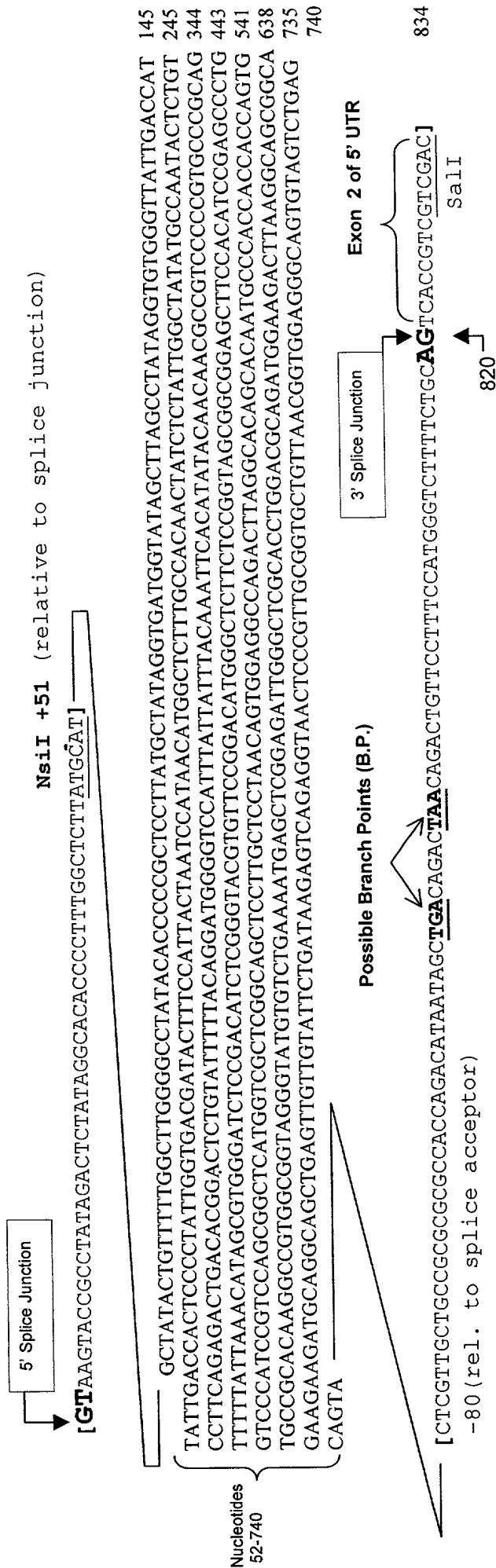


Figure 1B: Synthetic Oligo for substitution of nucleotides 52-740 of Intron A

[ATGCATCTCGTTGCTGCCGCGCGCCACCAGACATAATCGCTGACACACTGACAGACTGTTCCCTTCCATGGGTCTTTTCTGCAAGTCAACCGTCTGTCGAC]
NsiI -80
Changed to B.P. consensus
Changed to polypyrimidine consensus
SalI

Figure 1C: Deletion Mutant pCON3 Intron: 132 bp

NsiI
Branch Point

[GTAAGTACCGCCTATAGACTCTATAGGCACACCCCTTTGGCTCTTATGCAATCTCGTTGCTGCCGCGCGCCACCAGACATAATCGCTGACACACTGA]
CAGACTGTTCCCTTTTCTTTTGTGAGTCAACCGTCTGTCGAC]
Polypyrimidine Tract
SalI

Figure 2

1 ctgcagtgaa taataaaatg tgtgtttgtc cgaaatacgc gttttgagat ttctgtcgcc
61 gactaaaattc atgtcgcgcg atagtgggtt ttatcgccga tagagatggc gatattggaa
121 aaatcgatat ttgaaaatat ggcataattga aaatgtcgcc gatgtgagtt tctgtgtaac
181 tgatatacgcc atttttccaa aagtgatttt tgggcatacg cgatatctgg cgatacggct
241 tatatcgttt acgggggatg gcgatagacg actttggcga cttgggcgat tctgtgtgtc
301 gcaaatatcg cagtttcgat ataggtgaca gacgatatga ggctatatcg ccgatagagg
361 cgacatcaag ctggcacatg gccaatgcat atcgatctat acattgaatc aatattggca
421 attagccata ttagtcattg gttatatagc ataaatcaat attggctatt ggccattgca
481 tacgttgtat ctatatcata atatgtacat ttatattggc tcatgtccaa tatgaccgcc
541 atgttgacat tgattattga ctagtatta atagtaatca attacggggt cattagttca
601 tagcccatat atggagttcc gcgttacata acttacggta aatggcccgc ctctgacccg
661 cccaacgacc cccgccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata
721 gggactttcc attgacgtca atgggtggag tatttacggg aaactgcca cttggcagta
781 catcaagtgt atcatatgcc aagtcgggcc ccctattgac gtcaatgacg gtaaattggcc
841 cgctggcat tatgccaggt acatgacctt acgggacttt cctacttggc agtacatcta
901 cgtattagtc atcgctatta ccattggtgat gcggttttgg cagtacacca atgggcgtgg
961 atagcggttt gactcacggg gatttccaag tctccacccc attgacgtca atgggagttt
1021 gttttggcac caaaatcaac gggactttcc aaaatgtcgt aataaccccg ccccgttgac
1081 g^{caa}atgggc ggtaggcgtg tacggtggga ggtctat^{tata} agcagagctc gtttagtgaa
1141 ccg^ttcagatc gcctggagac gccatccacg ctgttttgac ctccatagaa gacaccggga
1201 ccgatccagc ctccgcggcc gggaaacggtg cattggaacg cggattcccc gtgccaaagag
1261 tgacGTAAGT ACCGCCTATA GACTCTATAG GCACACCCCT TTGGCTCTTA TGCATGCTAT
1321 ACTGTTTTTG GCTTGGGGCC TATACACCCC CGCTCCTTAT GCTATAGGTG ATGGTATAGC
1381 TTAGCCTATA GGTGTGGGTT ATTGACCATT ATTGACCACT CCCCTATTGG TGACGATACT
1441 TTCCATTACT AATCCATAAC ATGGCTCTTT GCCACAATA TCTCTATTGG CTATATGCCA
1501 ATACTCTGTC CTTGAGAGAC TGACACGGAC TCTGTATTTT TACAGGATGG GGTCCCATT
1561 ATTATTTACA AATTCACATA TACAACAACG CCGTCCCCCG TGCCCGCAGT TTTTATTAAA
1621 CATAGCGTGG GATCTCCACG CGAATCTCGG GTACGTGTTC CGGACATGGG CTCTTCTCCG
1681 GTAGCGGCGG AGCTTCCACA TCCGAGCCCT GGTCCCATGC CTCCAGCGGC TCATGGTCGC
1741 TCGGCAGCTC CTTGCTCCTA ACAGTGGAGG CCAGACTTAG GCACAGCACA ATGCCACCA
1801 CCACCAAGTGT GCCGCACAAG GCCGTGGCGG TAGGGTATGT GTCTGAAAAT GAGCTCGGAG
1861 ATTGGGCTCG CACCGTGACG CAGATGGAAG ACTTAAGGCA GCGGCAGAAG AAGATGCAGG
1921 CAGCTGAGTT GTTGTATTCT GATAAGAGTC AGAGGTAAGT CCCGTTGCGG TGCTGTAAAC
1981 GGTGGAGGGC AGTGTAGTCT GAGCAGTACT CGTTGCTGCC GCGCGCGCCA CCAGACATAA
2041 TAGCTGACAG ACTAACAGAC TGTTCTTTTC CATGGGTCTT TTCTGCAGtc accgtccttg
2101 acacgatgga gtcctctgcc aagagaaag^atg gaccctga taatcctgac gagggccctt
2161 cctccaaggt

Enhancer Region
(~600 - ~1081)

Pol II Promoter
(1081 - 1143)

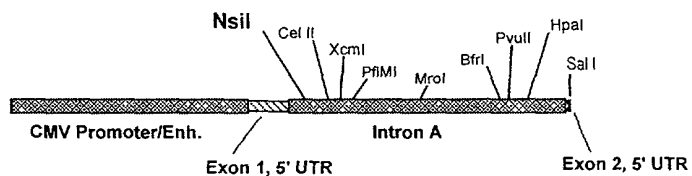
Exon 1 (5' UTR)
(1144 - 1264)

Intron A
(1265 - 2088)

Exon 2 (5' UTR,
Start of Trl.)
(2089 -)

Figure 3

Deletions Made Within Intron A of CMV IE1



DELETION	LENGTH *		
Nsi-Cel II	70 bp		Nsi - X Truncations
Nsi-XcmI	113 bp		
Nsi-PflMI	150 bp		
Nsi-MroI	345 bp		
Nsi-BfrI	578 bp		
Nsi-PvuII	609 bp		
Nsi-HpaI	663 bp		

DELETION	LENGTH *		
Hpa-PvuII	54 bp		Hpa - X Truncations
Hpa-BfrI	80 bp		
Hpa-MroI	314 bp		
Hpa-PflMI	516 bp		
Hpa-Cel II	590 bp		

* Following restriction enzyme digestion, blunting, religation

0997067404

Figure 4

Intron A Internal Deletion Mutants
(Transiently-Transfected 293 cells)

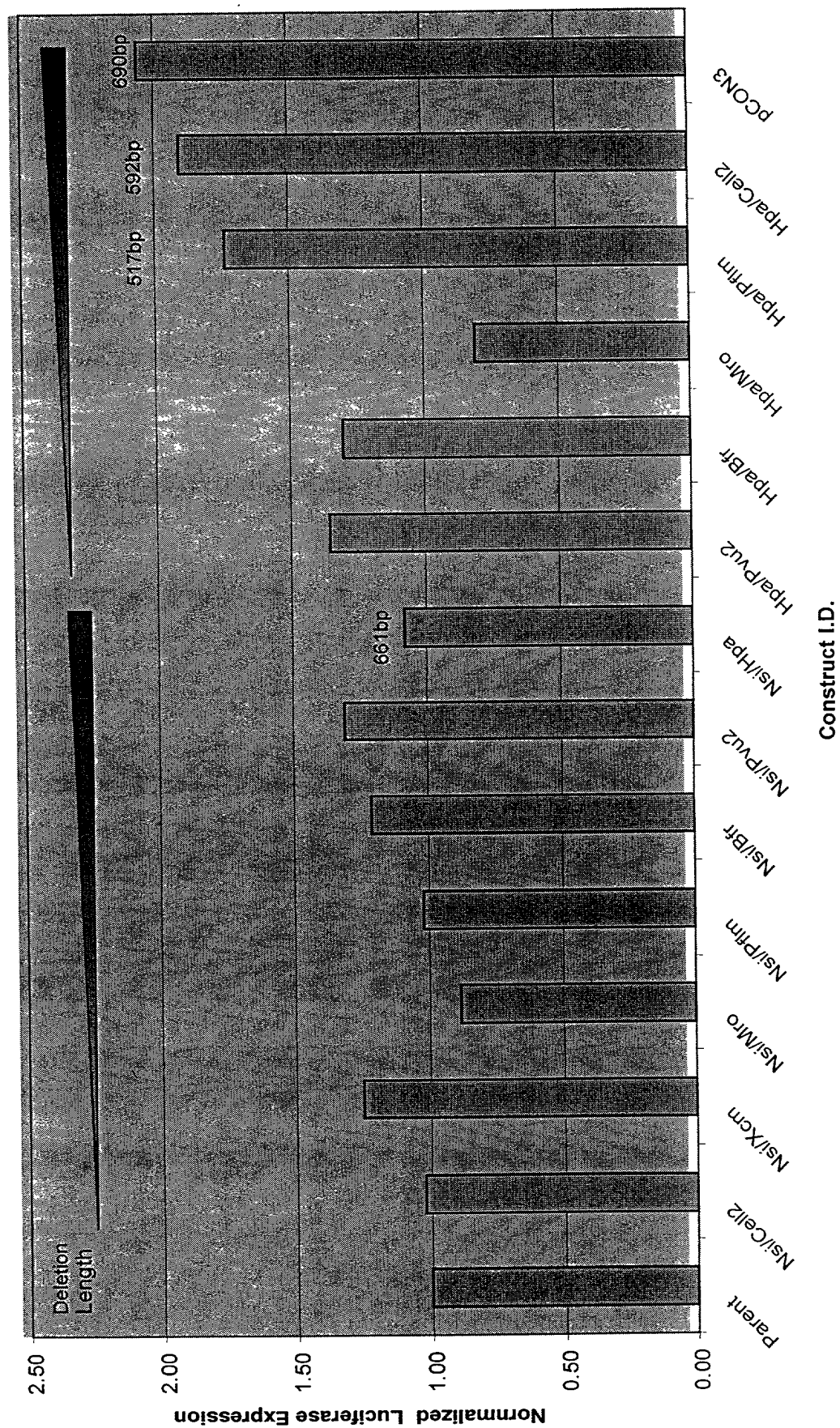


Figure 5A

1. Wild Type Rabbit β -Globin Sequence

GTTGGTATCCTTTTTACAGCACAACTTAATGAGACAGATAGAACTGGTCTTGTAGAAACA

Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTCTGACTTCTCTCCCCTGGGCTGTTTTCATTTTCTCAG

Branch Pt.

Polypyrimidine Tract

Figure 5B

2. Optimized Rabbit β -Globin Sequence

GTAAGTATCCTTTTTACAGCACAACTTAATGAGACAGATAGAACTGGTCTTGTAGAAACA

Splice Donor

GAGTAGTCGCCTGCTTTTCTGCCAGGTACTAACTTCTCTCCCCTCTCCTCTTTTCTTTTCTGCAG

Branch Pt.

Polypyrimidine Tract

Figure 6

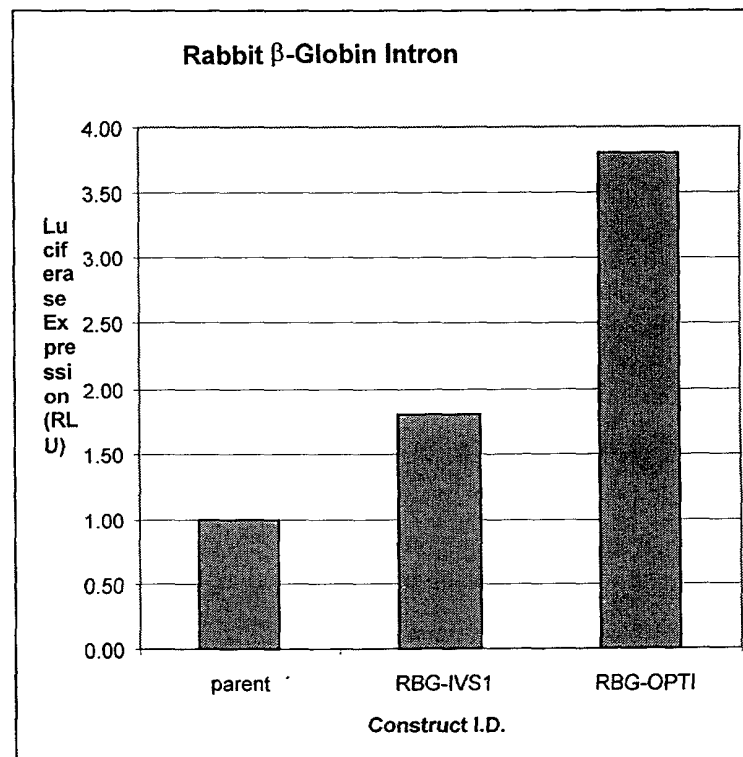
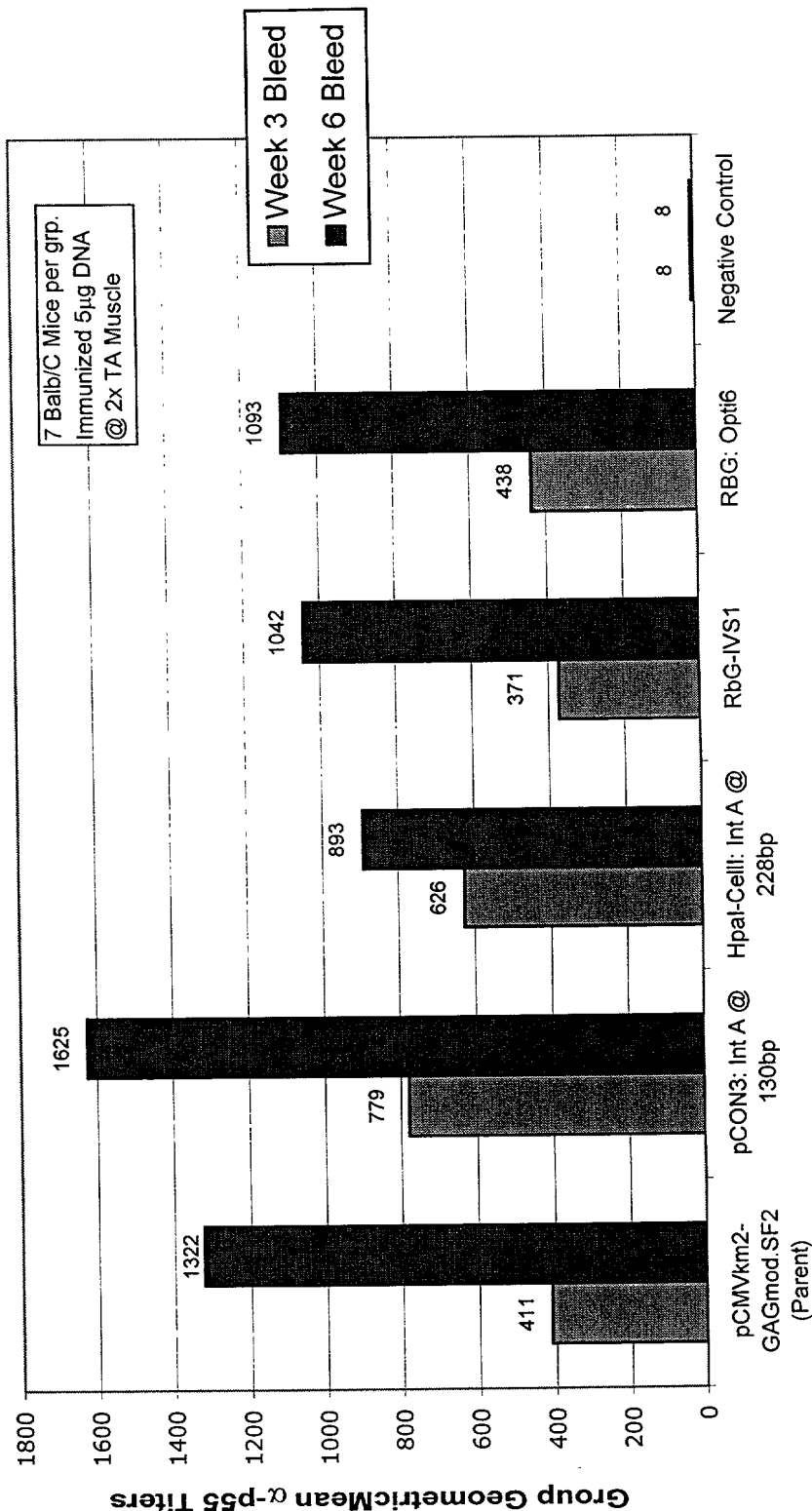


FIGURE 7

**In Vivo Immunogenicity of Plasmid Vectors
Containing Modified Introns**



Immunizing Vector DNA